Effect of Steriods on TNF-Alpha Expression in Whole Blood Cell Cultures of Individuals with Human Immunodeficiency Virus Infection

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Objective: To compare the Tumor Necrosis Factor-alpha (TNF-α) levels in HIV infected and uninfected healthy individuals and to evaluate TNF-α release in the presence of Vitamin D3, EB 1089 and methyl prednisolone in whole blood cell cultures of HIV infected and uninfected individuals.

Methods: Plasma TNF-α estimation: Blood was collected from healthy blood donors (n = 66) & HIV infected patients (n = 71) after their consent. Plasma was separated and stored at -70°C till TNF-α levels were measured.

Whole Blood cell assay: TNF-α release was evaluated in whole blood cell cultures of HIV infected (n = 13) and uninfected individuals (n = 7) with and without modulators such as Phytohaemagglutin (PHA) and Concanavalin-A (Con-A); Methylprednisolone sodium succinate, Vitamin D3 and EB1089 all of which have a potential affect on TNF-α release.

Cytokine assay: The TNF-α levels were estimated by sandwich ELISA using R&D systems kit.

Results: There was a wide range of values of TNF-α levels in individuals of both uninfected and HIV infected groups. There was a significant decrease in the plasma TNF-α levels in HIV infected as compared to uninfected group. Stimulation of whole blood cell cultures by Con A & PHA did not show significant differences in the TNF-α release between uninfected and HIV infected individuals. When stimulated whole blood cultures were incubated in the presence of methylprednisolone, vitamin D3 and EB 1089, only methylprednisolone showed significant difference in the inhibition pattern as compared to others.

Conclusion: There is a wide scatter of individual plasma TNF-α values in patients & normal individuals. TNF-α release in the whole blood assays of normal & HIV patients also showed a wide variation. Methylprednisolone exerted maximum inhibition in TNF-α release as compared to other modulators. Clinical follow up and longitudinal studies on TNF-α levels in patients could indicate its role in the progression of HIV to AIDS.

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41.002

A Prospective Study on Immune Restoration Disease in HIV-Infected Patients Following Successful ART at UMMC

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Approximately 10–40% of HIV-infected patients responding to antiretroviral therapy (ART) develop immune restoration disease (IRD). It is thought that patients experience clinical deterioration as a result of an inflammatory response to intact subclinical pathogens and/or residual antigens.

Objective: To determine the incidence and risk factors for the development of IRD in a cohort of severely immunosuppressed patients during successful treatment with ART (triple therapy) at the UMMC clinic.

Methods: Patients were monitored and clinical data characterized at weeks 0, 6, 12, 24 and 48 of ART from 47 patients with age, gender, ethnicity, CD4 T-cell count and percentage, and plasma HIV RNA.

Results: The incidence of IRD in our cohort was 27.7%, and the commonest IRD event was an exacerbation of symptoms (cervical lymphadenitis, lymphadenopathy and lymph nodes evolving into abscesses or cold abscess enlargement) associated with pre-existing infections with Mycobacterium tuberculosis (Mtb). Having a low baseline CD4 T-cell count and percentage was a risk factor for developing IRD. TB IRD occurred in patients who started ART within 6 weeks of TB treatment.

Conclusion: Patients with advanced disease initiating ART must be closely monitored in the first 6 months for development of IRD. In areas where MTB is endemic, TB IRD may occur frequently and lead to diagnostic and therapeutic challenges.

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41.004

Immunological Profiles of Immune Restoration Disease Presenting as Mmmcobacterial Lymphadenitis or Cryptococcal Meningitis

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Objectives: A proportion of HIV patients beginning antiretroviral therapy (ART) develop immune restoration disease (IRD). Immunological characteristics of IRD were...
investigated in a cohort of severely immunosuppressed HIV patients beginning therapy at University Malaya Medical Centre (UMMC).

**Design:** Peripheral Blood Mononuclear Cell (PBMC) were collected at weeks 0, 6, 12, 24 and 48 of ART from five patients experiencing IRD (3 with cryptococcal and 2 with Mycobacterium tuberculosis [Mtb] disease) and eight non-IRD controls who had begun ART with CD4 T-cell counts of <100/µL.

**Methods:** T cells producing interferon-gamma (IFN-γ) were quantified by ELISpot assay after stimulation with purified protein derivative (PPD), 6kDa early-secreted antigenic target (ESAT-6), Cryptococcus neoformsans or Cytomegalovirus (CMV) antigens. Plasma IgG antibody to these antigens were assayed by ELISA. Proportions of activated (HLA-DRhi) and regulatory (CD25+CD127lo and CTLA-4+)-CD4+ T-cells were quantitated by flow cytometry.

**Results:** IRD patients displayed elevated IFN-γ responses and/or plasma IgG antibody to PPD, but none responded to ESAT-6. Cryptococcal IRD was associated with IFN-γ and antibody responses to cryptococcal antigen. Proportions of activated and natural regulatory CD4+ T-cells (Treg) declined on ART, but remained higher in patients than healthy controls. At the time of IRD, proportions of activated CD4+ T-cells and Treg were generally elevated relative to other patients.

**Conclusions:** Cryptococcal and Mtb IRD generally coincided with peaks in the proportion of activated T-cells, antigen-specific IFN-γ responses and reactive plasma IgG. IRD did not reflect a paucity of Treg.

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41.005

Human Immunodeficiency Virus (HIV) Cervicovaginal Shedding During the Menstrual Cycle in Seropositive Women Followed at a Specialized Care Center in São Paulo

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The sexual route is the main means of transmission of the human immunodeficiency virus (HIV). With the increasing numbers of HIV-infected women, the investigation of particular biological features of HIV infection in the genital tract has become more important. To evaluate HIV genital shedding during the menstrual cycle, we collected cervicovaginal lavages (CVL) from 17 women, assisted at an HIV outpatient clinic in São Paulo, in different hormonal phases during 2 cycles. HIV-RNA and proviral DNA shedding were quantified using RT-PCR and a TaqMan real-time PCR assay, respectively. In addition, patients were screened for genital coinfections and had their HIV plasma viral loads and CD4+ cell counts assessed. Cell-free HIV-RNA and proviral DNA shedding were found in 18.8% and 31.3% of women. All patients who shed HIV-RNA were also shown to present detectable proviral DNA in their CVL, including one woman with undetectable HIV plasma viral load. No significant difference in viral shedding was seen among menstrual cycle phases. Six patients from the cohort, who exhibited genital coinfections previous to admission to the study, had their HIV genital shedding compared at time of coinfection and after its resolution. In two of them proviral DNA shedding was higher at the time of coinfection, caused by Streptococcus sp and Ureaplasma sp. No cell-free HIV-RNA shedding was detected in coinfected patients. Our results may contribute to the understanding of HIV sexual infectivity from women and emphasize the need for adherence to protected sexual practices in order to avoid viral transmission.

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41.006

HIV Cervicovaginal Shedding among Postmenopausal and Fertile Women from Sao Paulo, Brazil

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**Background:** Few studies have focused on modifications in the genital tract of postmenopausal women and their association with HIV shedding. In this cross-sectional study HIV-RNA shedding was compared between fertile and postmenopausal women followed at a specialized centre in Sao Paulo, Brazil. Correlations between viral shedding and HIV viral loads and CD4+ lymphocyte counts were also investigated.

**Methods:** 146 HIV-infected women [73 postmenopausal (PM)/73 fertile(F)] were enrolled at the University of Sao Paulo Medical School. Postmenopausal women referred a mean duration of 8.17 years since menopause. CD4+ cell counts were assessed by flow cytometry and HIV-RNA quantified in plasma and in cervicovaginal lavages (CVL) using Cobas Amplicor HIV-1 Monitor Ultrasensitive Test. LiCl was introduced into the CVL buffer and measured before and after sample collection to determine CVL dilution for each specimen.

**Results:** The prevalence of HIV-RNA shedding was similar in both groups (PM: 17.8%, 95% CI 9.8—28.5 vs. F: 22%, 95% CI 13.1—33.1). Likewise, mean intensity of shedding was shown not to differ between groups (PM: 2.18log/mL, F: 2.25log/mL). Plasma viral loads were detectable in 34.2% of postmenopausal, as compared to 42.5% of fertile women (p = 0.395). Three patients (2 PM/1 F) exhibited HIV shedding in the absence of detectable viremia. HIV plasma viral loads and cervicovaginal shedding were significantly correlated in both groups (PM: r = 0.658, F: r = 0.684, p < 0.01). CD4+ cell counts were negatively correlated to HIV shedding (PM: r = −0.250, F: r = −0.248, p < 0.05).

**Conclusions:** Despite changes that occur in the vaginal mucosa of menopausal women, HIV shedding does not seem to be significantly influenced by this state. Plasma viral loads and CD4+ cell counts are correlated to HIV shedding, but these factors alone do not fully predict HIV loads in CVL,